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Dear Sirs

Re : **RESPONSE TO THE 2nd WRITTEN OPINION -**
PCT International Application No. PCT/IN03/00289
dated 27 August 2003 (27.08.2003)
Applicant : LUPIN LTD. et al
Title : Herbal Extract Comprising A Mixture Of Saponins Obtained From Sapindus Trifoliatus For Anticonvulsant Activity.
Priority Date : 28 August 2002 (28-08-2002)
Agent's File Reference : FPAA335PCT

Please refer to the 2nd written opinion dated July 23, 2004 issued on the above. On the statement with regard to the novelty, inventive step and industrial applicability we submit as under :

The present invention relates to an anticonvulsant composition comprising extract of the pericarp of the fruit of *S. trifoliatus* with defined amount of hederagenin wherein the activity of the composition is mediated by affinities for defined receptor sites.

Regarding the cited art D1 (Chaturvedi et al), it is respectfully submitted that the document indicates triterpenoids including hederagenin for anti-convulsant activity. There is no mention of extraction of the pericarp of the fruit of *S. trifoliatus* with the amount of hederagenin as indicated in the present invention. It is further submitted that the present invention does not claim hederagenin as such or its activity. The invention relates to anti-convulsant pharmaceutical composition comprising an extract of pericarp of the fruit of *S. trifoliatus* containing 0.001 to 1% of hederagenin and pharmaceutically additives wherein the composition acts specifically by having certain binding affinities as defined in the text and claims. The present invention does not relate to anti-convulsant activity of hederagenin. On the other hand it relates to the anti-convulsant activity in this saponin mixture extracted from fruit of *S. trifoliatus* which contain the hederagenin in a defined amount. The attention of the Ld. Examiner is drawn at page 17 wherein it is indicated that acid hydrolysis of the extract from pericarp of fruit of *S. trifoliatus* yielded only one aglycone which was identified as hederagenin. Accordingly, the hederagenin indicated in the present invention is always in reference to saponins as derived from pericarp of fruit of *S. trifoliatus*. Hence the present invention does not indicate or claim the role of hederagenin per se but that derived from pericarp of fruit of *S. trifoliatus* which is neither taught nor motivated from the cited art. Moreover, the mode of action of the present composition relating to binding affinities for defined receptor sites is also indicated in the present invention which is not taught by the cited art. Accordingly the

present invention ought not to be regarded as anticipate by the cited art since reference to hederagenin at all steps as being made only for quantification of saponins as hederagenin and not hederagenin per se.

It is further submitted that the present invention reports for the first time, that the ED_{50} of the aqueous extract is only 7.72mg/kg i.n. as against 100 mg/kg i.p. of pure hederagenin and its methyl ester acetate as reported in the above paper. Therefore, the composition comprising the aqueous extract of the present invention shows much better anticonvulsant activity as compared to the pure compounds of the cited art.

As to the cited art D 2 (EP 0 767 177 A1) relates to the study of hederagenin and its derivatives (NOT SAPONINS glycoside derivatives of hederagenin) for a pharmaceutical composition for therapy of nephritis.

Example 1 of this cited art describes the sulphuric acid hydrolysis of the extract of the pericarp of *Sapindus mukorossi* to obtain hederagenin which has been administered as a pharmaceutical composition containing 0.1-99.5 % of it in a medicinally acceptable non-toxic inert carrier. Hence, the teaching of D2 does not fall into the scope of Claim 1 since the said claim of the present invention does not claim the extract of the pericarp of the fruit but an anti-convulsant composition comprising extract from pericarp of fruit of *S. trifoliatus*. That such a composition with the extract having defined amount of hederagenin in a pharmaceutical composition could act as anti-convulsant with specific binding affinities for defined receptor sites is neither taught nor motivated from the cited art.

D3 (Patent Abstracts of Japan and JP 02 262510) teaches a dentrifice composition with antiinflammatory activity comprising an extract containing hederagenin extracted from pericarp of *Sapindus mukorossi* and extract as such.

The present invention on the other hand involves the use of aqueous extract of *Sapindus trifoliatus*, which is a different species. Further anticonvulsant activity of the said extract in a composition and the mechanism of action is claimed and disclosed in the present invention. No anti-inflammatory activity is claimed. Taking cue from this cited art no ordinarily persons skilled in the art could arrive at the present invention comprising a pharmaceutical composition for anti-convulsant activity with specific binding as affinities for specific receptor sites and comprising extract of pericarp of fruit of *S. trifoliatus* comprising hederagenin in a defined amount.

D4 (Higuchi, R.. et al.) teaches isolation of saponins from *Akebia quinata* containing hederagenin as aglycone. No activity has been reported neither is there any mention of the amount of hederagenin in the extract of the pericarp of *Sapindus trifoliatus*. In the present invention composition comprising the aqueous extract of the pericarp of *Sapindus trifoliatus* having anticonvulsant activity is described and claimed. No process for the isolation of compounds. Further, the species *Akebia quinata* is totally different from *Sapindus trifoliatus*.

D5 (Kanchanapoom et al.) teaches the isolation of various saponins from *Sapindus emarginatus* containing hederagenin as the aglycone. No anti-convulsant activity has been reported. The reported activity in Thai traditional medicine pertains to the pericarp only.

In the present invention the anticonvulsant activity of the aqueous extract of the pericarp of *Sapindus trifoliatus* is demonstrated with respect to binding affinities for defined receptors. No process for the isolation of compounds is claimed.

It is further submitted that the compounds present in the extract of pericarp of *Sapindus trifoliatus* are saponins which contain hederagenin as the aglycone. The saponins were estimated after hydrolysis as hederagenin by using HPLC. It is submitted that the activity of a composition comprising the said saponins has not been reported in any of the references. The applicants report the anticonvulsant activity in the saponin mixture present in the extract of *S. trifoliatus* with reference to affinities for specific binding sites for the first time.

Such anticonvulsant activity is mediated via binding affinities for the receptor sites viz. GABA-A agonist site, Glutamate AMPA site, Glutamate Kainate site, Glutamate-NMDA agonistic site, Glutamate NMDA glycine (strychnine insensitive) site and sodium channel (site 2). The activity of saponins from extract of pericarp of *Sapindus trifoliatus* comprising hederagenin as the aglycone through such binding sites have not been taught or motivated by any of the cited art.

Thus the cited art alone or in combination does not teach or motivate the present invention for anti-convulsant pharmaceutical composition comprising extract from pericarp of *Sapindus trifoliatus* with defined amount of hederagenin, said composition being suited for nasal administration and its activity being mediated by affinities for defined the receptor sites.

The above may kindly taken into consideration and a positive examination report may kindly be issued.

Yours faithfully,

Ganguli

Dr. Sanchita Ganguli
Applicant's Agent